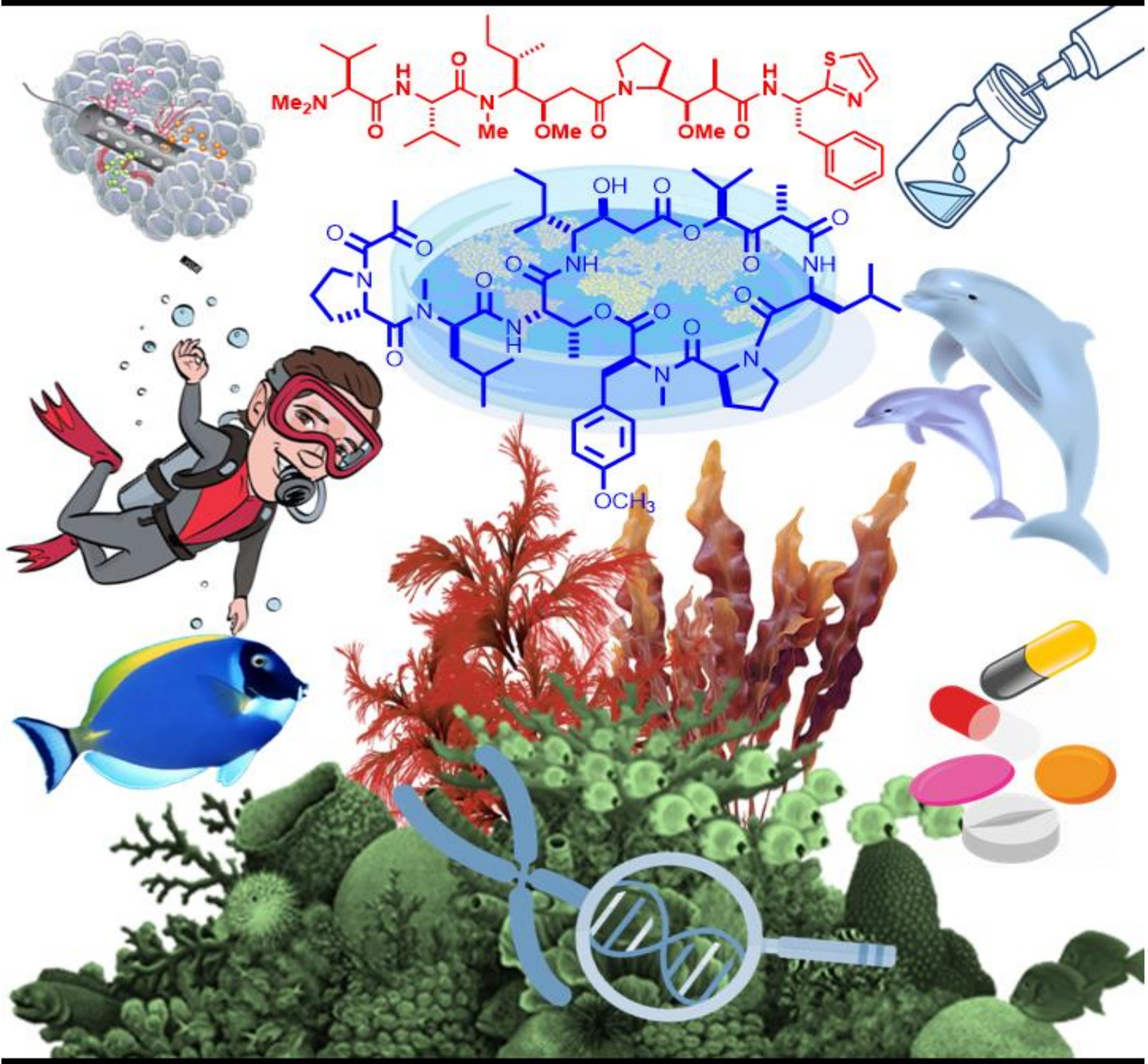
Marine Drugs

2021 – 2022

{Exam 1 With Answers}



Dr. Omar M. Sabry

☆ يتكون الامتحان من (**5**) اسئلة في عدد (**10**) صفحة

☆ اجابة السؤال الأول في ال **ANSWER SHEET** المخصص لذلك

Question No. I **{25 Marks}**

Choose the correct answer for each question. Mark your choices in the answer sheet.

1- Drug discovery scientists were increasingly turning to Marine Natural Products {MNPs} as a new source in the search for biologically and pharmacologically active compounds due to

a- Drug resistance b- Appearance of new diseases

c- Oceans and seas occupy 70% of earth surface **d- All of the above**

2- Marine Natural Products science is a modern science started in the late 1970th because

a- No SCUBA diving before 1970th b- No submarines before 1970th

c- a and b d- No snorkeling

3- If divers are going deeper than 100 feet in Ocean's water they will suffer from

a- Azotemia b- Embolism c- Death **d- All of the above**

4- The marine drug isolated from puffer fish and 3000 times more active than morphine as pain killer

a- Tetrodotoxin b- Stypoldione c- Conotoxin d- None of the above

5- The marine drug inspired from Halichondrin B by structural trimming used for treating breast cancer

a- Halichondrin A **b- Eribulin** c- Aplidin d- Halogenated monoterpenes

6- Marine compound from cone snail acts as Ca^{++} channels blocker on the neurons in the spinal cord

a- ω-conotoxin b- Jellyfish toxins c- Brevetoxin B d- Trabectedin

7- The marine compound that acts as Vasoconstrictor and can treat hypotension

a- Ciguatoxin **b- Maitotoxin** c- Aplysiatoxin d- Pseudoterosine A

8- Gene isolation and transfer to *E. coli* to produce the active constituents *in-vitro* is known as

a- Biotechnology b- Gene hunting c- Gene expression d- Gene gun

9- The marine compounds that can be used as lead drug for treatment of cardiac arrhythmia

a- Polypeptides **b- Jellyfish toxins** c- K^+ channel blockers d- Na^+ channel influxs

10- The marine drug isolated from an Ascidian and acts as DNA alkylating in colorectal cancer

a- Halaven **b- Trabectedin** c- Brentuximab d- Pseudopterosine A

11- Marine drug from soft coral has COX I and II inhibitory activity in skin trauma {Anti-inflammatory}

a- Psudoterosine A b- PUFAs c- Aplysiatoxin d- Penta-bromo pseudalin

12- Preserved in lyophilized state at low temperature and constituted directly before use

a- Ziconotide b- Brentuximab c- Therapeutic polypeptides **d- All of the above**

13- Large number of M.O. growing in short time

a- Nutrient agar media b- Nutrient blood media c- MacConkey agar **d- HTPS**

14- Inhibits DNA polymerase and used for treatment of Herpes and Chickenpox

a- Cytarabine **b- Vidarabine** c- Dolastatin 10 d- Tetrodotoxin

15- Depsipeptide marine drug that induces apoptosis in Pancreatic cancer

a- Dolastatin 10 **b- Aplidin** c- Trabecetedin d- Dolastatin 15

16- A drug inspired from Dolastatin 10 to increase selecticity and reduce toxicity in treating lymphoma

a- Brentuximab {ADCETRIS} b- Doastatin 15 c- Eribulin d- Trabectedin

17- Tumor promoter in large dose, and in small dose it induces cell death produced be cyanobacteria

a- Polyhalogenated monoterpenes b- Curacin A **c- Aplysiatoxin** d- kahalilide

18- A compound from a brown alga that blocks sodium channels and kills snails

a- Stypoldione b- Pentabromo-pseudalin c- Prialt d- Halneuron

19- Alternative source in shortage of marine drugs supplying source

a- Aqua-culturing, b- chemical synthesis c- biotecnology **d- Any of the above**

20- Technology uses plants to produce pharmaceuticals like vaccines and antibodies

a- Plant biotechnology **b- Molecular farming** c- Plant cell culture d- Plant tissue culture

21- Every triplet of nucleotides in a nucleic acid sequence specifies a single amino acid

a- Gene b- Nucleotide sequence **c- Codon** d- Genetic code

22- Indispensible tools of Recombinant DNA Technology

a- Lysins **b- Restriction enzymes** c- Ligases d- None of the above

23- Gene finding that contributes to biosynthesis of certain bioactive compounds

a- Gene hunting b- Gene gun c- Gene sequencing d- Gene transfer

24- Works as a natural genetic engineer

a- *E. coli* b- Yeast **c- *Agrobacterium tumifaciens*** d- *Alteromonas*

25- Produced on a RNA template by the action of reverse transcriptase

a- mRNA b- tRNA rRNA **d- cDNA**

Question No. II **{15 Marks}**

Rationalize the following statements {in summary}

1- Marine drug discovery scientists must know a simple background about marine creatures taxonomy.

As during marine samples collection each class of marine organisms contains certain chemical groups of active constituents with specific pharmacological activities e.g. Blue green algae contain polypeptides showing promising cytotoxic activity against cancer cells. Scientists will avoid samples known to contain inactive compounds.

2- Marine organisms are extracted with organic solvents for screening and isolation of marine drugs

2ry metabolic compounds are lipophilic {hydrophobic} compounds. In extraction step, sample homogenized then extracted with the suitable organic solvent e.g. ethanol, methanol, CH_2Cl_2, acetone and acetonitrile to dissolve the pharmacologically active organic compounds. If we use water for extraction, it will not dissolve these bioactive compounds.

3- Brine shrimp toxicity or Goldfish toxicity is an important step in isolation of bioactive marine drugs

Brine shrimp toxicity or fish toxicity are important steps in isolation of bioactive marine Drugs. If the extract or the fraction is toxic to {Brine shrimp - snails - Gold fish}, extract must contain strong pharmacologically active compound{s} that is/are toxic to Brine shrimps in large dose. But when used in diluted concentration, it can treat certain disorders e.g. Sodium channel blockers in large dose can cause respiratory paralysis and death to Brine shrimp, but in small doses can be used as local anesthetic or pain killer in chronic inflammation {e.g. Tetrodotoxin}.

4- Many bioactive marine compounds fail to be approved as drugs or medicines

Many marine natural compounds failed in clinical trials phases as they may have unresolved problems in instability, insufficient supplying source, high toxicity and difficulty in total synthesis or semi-synthesis.

5- Marine drugs are of wide chemical diversity

The nature of compounds characterized from marine organisms are diverse and represent many different structural classes, including linear Peptides {Dolastatin 10}, cyclic Peptides {ω-Conotoxin}, Cyclic depsipeptides {Aplidin}, Nucleosides {Ara C - Ara A}, Polyether or Polyketides {Halichondrin B}, Alkaloids {Trabectedin}, PUFAs {Omega 3 fatty acids} and terpenoids {Pseudoterosine A}.

6- Marine polypeptides are considered as the drugs of future

They have highly specific mode of actions, needed in lower doses, have reduced risk of toxicity, used for bacterial infections, have diverse biological activities e.g. anti-inflammatory activity, anti HIV activity, antibiotic activity, and anti-cancer activity.

7- During extraction, purifications, isolation and medical use of polypeptides, we have to be cautious

- They will be damaged {de-naturated} by high PH, low PH and high temperature {higher than 30 ^{0}C}. Extraction of Polypeptides from marine creatures must be carried out as soon as possible or from alcohol preserved organisms to inhibit enzyme activity and to prevent hydrolysis of polypeptides.
- Medical Polypeptides {Ziconotide} must be preserved in a lyophilized state at low temperature in a refrigerator and constituted in their solution for injection directly before use to avoid hydrolysis.
- Peptides with therapeutic value can't be administered orally nor by I.M injection as Plasma membrane restricts their movement across intestinal epithelial cells and also, they are degraded by protease enzyme.

8- Marine drugs discovery scientists must know how to culture marine organisms in the laboratory.

The true source of most of the isolated Marine Natural Products compounds is the symbiotic microorganisms rather than the host. So, it is important for Marine Natural Products scientist to know how to culture marine micro-organisms to isolate the bioactive compound or even the gene cluster from the true producer. Upon carrying out microbial culturing of the sea water micro-organisms, only 0.1% of the marine microorganisms will grow {due to differences in the conditions between sea water and nutrient media}. HTPS {High Throuput Screening Technique} can be used for growing microorganisms in short time and high numbers.

9- ESIMS and FABMS are more preferable MS ionization techniques than EIMS in analysis of marine bioactive peptides

ESIMS {Electro Spray Ionization Mass Spectroscopy} and FABMS {Fast Atom Bombardment Mass Spectroscopy} are more preferable MS techniques in determination of the molecular weight and the sequence of amino acids as they are soft techniques giving gradual fragmentation of the peptides and cyclic depsipeptides. So, we can know the molecular weight and predict the amino acid constituents and their sequence in the polypeptide easily.

EIMS {Electro Impact Mass Spectroscopy} is a tough technique giving sudden and complete fragmentation of the cyclic peptides {e.g. complete damage of amino acids}. So, we can't predict the amino acids and their sequence.

10- Scientists may need to carry out structural trimming of some marine natural products

To decrease side effects, increase selectivity, decrease toxicity or increase bioavailability e.g. Structural trimming of Halichondrin B was carried out to obtain Eribulin.

11- Omega-3 fatty acids esters are more preferable than omega-6 fatty acids as cardioprotective drugs

Omega-3 fatty acids esters share in biosynthesis of the useful ecosonoids

Omega-6 fatty acids share in biosynthesis of the harmful ecosonoids

12- Some scientists are giving the name blue green algae to cyanobacteria, while others are refusing to change the name?

Scientists debate about the nomenclature of Cyanobacteria to be cyanobacteria or blue green algae? Some scientists named them Cyanobacteria as the cell wall consisting of peptidoglycans {like bacteria} and a mixture of pigments. Other scientists named them blue green algae as they contain chlorophyll {green pigments} and Phycobillin {Blue pigments} carry out photosynthesis using chlorophyll.

13- Scientists had no other choice than to carry out total synthesis of Eribulin {Halaven}

Halichondrin B showed a promising activity against breast cancer. When they tried to produce it: 1- Directly from Sponge the quantities were insufficient 2- Aqua culturing it was difficult to get the

quantities required for the market 3- by microbial culturing they couldn't grow the microbes from the sponge as only 0.1 percent will grow 4- by biotechnology they couldn't get the gene cluster from the sponge nor from the microorganisms as it is synthesized by many micro-organisms so finally they carried out total synthesis and during synthesis they carried out modification of the structure of Halichondrin B to Eribulin by structural trimming to reduce toxicity by decreasing lipophilicity

14- In biotechnology, genetic code is universal

Same code is used by bacteria, plants and humans. gene encoding a plant protein can be inserted into a bacterium.

15- Palytoxin can be used as an alternative to digoxin {digitalis}

As it acts by inhibition of Na^+/K^+ ATPase {increases sodium influx through sodium pump} also increasing Ca^{++} influx inside cells leading to muscle contraction {Cardiotonic activity}.

Bonus Question

From ^{1}HNMR spectra, we can know the number of amino acids in a polypeptide **{1 Marks}**

Number of amide groups O=C-N-**H** + methyl amide groups O=C-N-**CH_3** + 1

Question No. III **{15 Marks}**

Match the scientific term number in **column A** with the letter equivalent to definition in **column B.** Record the chosen letter in the following table

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
d	g	j	L	a	o	m	k	b	c	n	h	f	e	i

Column A	Column B
1- Marine toxins	a- Identifies structure of the compound and its stereochemistry
2- Bioassay-guided fractionation	b- Phage lysins will facilitate the entrance of antibiotic to resistant bacterial cell causing bacterial cell death
3- Elemental analysis	c- Fluorescent compound can be used for determining cardiac output
4- Infrared spectroscopy (IR)	d- Sea food poisoning
5- X-ray crystallography	e- Biolistic particle delivery system
6- Depsipeptides	f- Cut up and join together genetic material
7- Trabectedin {Yondelis™]	g- Testing the crude extract or preliminary fractions from chromatography in an assay or multiple assays
8- Marine biotechnology	h- Cleaves DNA at specific sites
9- Overcoming Bacterial Resistance	i- Modern approach to isolate the DNA of microbial communities directly from their natural environments
10- Coelenterazine	j- Percentage elements in the compound
11- Genetic code	k- Culture-independent method (Marine biotechnology) to explore the untapped reservoir of marine microbial chemical diversity
12- Endonuclease	l- Gives an idea about functional groups e.g. OH, CH aromatic, C=O groups

13- Recombinant DNA technology	m- Semi-synthetic from Safracin B
14- Gene guns	n- The sequence of nucleotides in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
15- Metagenomics	o- Chain of amino acids alternating with carboxylic acids

Question No. IV **{10 Marks}**

Differentiate between the following:

1- Snorkeling and SCUBA diving as methods for marine sample collection

- Samples of marine organisms near shores are collected by hand or by Snorkeling.
- Samples of marine organisms up to 100 feet can be done by SCUBA diving.

2- Tetrodotoxin {Halneuron}and ω-Conotoxin as pain killers

Tetrodotoxin was isolated from Puffer fish. The actual producer is certain symbiotic bacteria {Pseudoalteromonas gram –ve M.O}. Tetrodotoxin acts as a very strong sodium channel blocker. It is converted into a pain killer medicine Halneuron™ by WEX Pharmaceuticals Canada. It is 3000 times more active than morphine.

ω-conotoxin block voltage-gated calcium channels in neurons. The synthetic analogue of ω-conotoxin {Ziconotide - Prialt} was produced and marketed now in year 2004. It is used to treat severe chronic pain in people who cannot use or do not respond to standard pain-relieving medications. It is 1000 times more active than morphine.

3- Ribosomal polypeptides {RPPs} and Non Ribosomal polypeptides {NRPPs}

Ribosomal polypeptides: Polypeptides biosynthesized by the routine transcription route {DNA contains gene cluster responsible for biosynthesis of peptides – DNA gives mRNA by translation. mRNA by translation gives polypeptides.

Non-ribosomal polypeptides {NRPP}: No gene cluster responsible for the biosynthesis of Polypeptides was found. It is said that: "NRPP are biosynthesized by a non-routine route which is constructed by Non ribosomal polypeptide synthases.

4- Polypeptides containing aromatic amino acids and Polypeptides without aromatic amino acids

UV spectroscopy: Aromatic amino acids absorb electromagnetic waves in UV range as they have conjugated systems λmax more than 260 - 280 nm.

IR spectroscopy: C-H aromatic at 3000 - 3200 cm^{-1}

^{1}HNMR: Aromatic protons chemical shift range 6.8 – 8.2 ppm

^{13}CNMR: Aromatic carbons at 110 – 150 ppm

5- HPLC/PDA and HPLC/UV in isolation and purification of marine polypeptides

PDA or DAD or PDAD (Photo Diode Array Detector) consists of 400 silicon chips (detectors). It can be used for a wide range of lambda max)200 - 800 nm). If there are 6 compounds in the mixture to be analyzed with lambda max 220, 250, 270, 290, 320 and 360 the PDAD will detect all of them. but if I have a single wavelength UV adjusted at 270 it will detect one compound only. If I have a variable wavelength UV adjusted at 270, 290 and 320 it will detect these 3 compounds only. Other compounds will not be recognized by the UV detector. Also, PDA or DAD detects peak purity while traditional UV Detector can't.

Question No. V **{15 Marks}**

1- A pharmaceutical company announced for a research project in marine drug discovery with 1,000,000 EUR grant value . Give titles and brief summary of each of the 2 proposals that you will submit?

(10 Marks)

1. Medicines from marine toxins: Like what had been done with tetrodotoxin

2. Using Bioassay guided isolation of bioactive compounds from marine organisms.

3.New medicines from marine biotechnology: Like what had been done with omega-3 with 7 unsaturation from diatoms and what had been done with Coelentrazine as a diagnostic element in cardiac output

4. Monoclonal antibody conjugates for marine drug targeting: Like what had been done with Brentuximab

5. Chemical synthesis of marine Drugs: Increasing selectivity and reducing toxicity by structural trimming like what had been done with Halichondrin B and Eribulin.

6. New microbial culturing techniques to encourage the growth of un-culturable microorganisms using new culturing media

7- Marine Drugs for Cancer from Cyanobacteria: Like what had been done with Dolastatin 10 as Cyanobacteria is rich in polypeptides and depsipolypeptides

8- Culturing of symbiotic microorganisms growing on Tunicates, Jellyfish and sponges: Like what had been done with bacteria producing Safracin B a precursor of Trabectedin

9- Isolation of gene clusters of Un-culturable marine microorganisms to discover new drugs

10- Marine Chemical Ecology as a source for new compounds with promising biological activity: Like what had been done with Omega conotoxins and ziconotide

2- Why was marine drugs course useful/unuseful for you? (5 Marks)

It is important for Drug discovery scientists to study Marine Natural Products branches. As after understanding the main ideas of each marine branch, they can inspire and discover new drugs from each branch.

A- Marine toxins: Toxic compounds isolated due to food poisoning e.g. Tetrodotoxin, a strong sodium channel blocker converted to a pain killer drug {Halneuron}. Halneuron is 3000 times more potent than morphine.

B- Marine Biopharmaceuticals: Bioactive compounds isolated for drug discovery e.g. Halichondrin B as anti-mitotic in breast cancer isolated from *Halichondria* sponge. Structure of Halichondrin B was modified {Structurally trimmed giving Eribulin - Halaven^{TR}} to decrease side effects and toxicity.

C- Marine Chemical Ecology: Interaction of marine creatures with each other e.g. Cone snail ω-conotoxin and paralysis of fish {Blocking Ca^{++} channels of the neurons in the spinal cord showing strong pain killing activity in chronic pain}.

D- Marine Biotechnology: Isolation of the gene clusters responsible for biosynthesis of certain bioactive compounds and transferring them to yeast, E. coli or to plant cells {transgenic cells} to produce large quantities of the active constituents in-vitro.

e.g. Transfer of gene cluster from diatoms producing omega-3 fatty acids with 7 un-saturations to E- coli.

Marine Drugs!

It is an Easy and Useful Topic

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